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STRESS REVERSIBLY AFFECTS IMMUNITY IN RATS

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ABSTRACT

This study investigated the effects of warm water swim stress and its withdrawal on the immunity of rats. Thirty-two adult male Wistar rats, average body weight (BW) 191.84 ± 1.61g grouped into four groups (n=8) were used for this study. Group A was the control and was not exposed to any particular stress; Group B rats were made to swim in warm (34 ± 0.5 ^{o}C) water for 3 minutes/day; and Group C rats in warm (37 ± 0.5 ^{o}C) water for 6 min/day; while Group D rats were made to swim in warm (40 ± 0.5 ^{o}C) water for 12 min/day. These continued for three weeks, after which each group was divided into two Sub-Groups (n_{SG}=4). Rats in one subgroup from each of the four groups were sacrificed 24 h after the last day of the 3 weeks of swim-stress, while the other sub-group from each of the four groups were left for 3 more weeks (to recover from the stress) before they were sacrificed. The WBC, CD4, and Differential WBC counts in the test and control groups were compared using independent-sample t-test. The results showed that the stress in groups B and C was moderate and significantly boosted the rats' immune components, while the stress in group D was severe and significantly reduce the rats' immunity. However, these changes were reversible (although not completely) upon stress withdrawal for three weeks.

KEYWORDS: Stress, immunity, blood, WBC, CD4, rat, differential WBC

INTRODUCTION

Various quantity and/or types of pressure (large or small; physical or psychological) could impose stress of different degrees on different individuals (Selye, 1950; Viner, 1999; Keil, 2004). Mild to moderate stress can be motivational and improve performance. In fact, some stress may help the body to prepare for certain challenges (Selye, 1950; Viner, 1999). However, a considerable amount of literature have documented it that too much of stress lead to physical, mental, and emotional (i.e. health) problems (Coren, 2008). In other words, sever and prolonged (unlike moderate) stress could breakdown mind and body systems.

Since stress is able to affect the state of health, it is reasonable and straight forward to believe that stress is capable of affecting or influencing the protective functions of the immune system. If stress would affect the immune system (Segerstrom and Miller, 2004; Herbert and Cohen, 1993), then: "What becomes of stress's effects on immunity after stress withdrawal?" "Will the effects be permanent or reversible?" If reversible, "how long will the required unaided recovery period be?" "Will the recovery be complete?" All of these make important research questions that had neither been answered nor previously thoroughly explored.

This research, therefore, evaluated the effects of graded amounts of stress and different durations of stress, as well as their withdrawal on immune system of adult male Wistar rats, with the aim of providing answers to the above research questions.

MATERIALS AND METHODS

Thirty-two adult male Wistar rats, average body weight (BW) 191.84 ± 1.61g, obtained from the animal house section of the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria were used for this study. The animals were allowed to acclimatize over a period of ten days.

Experimental Design and Animal Treatment
The thirty-two rats were randomly grouped into four (Group A, B, C and D, n = 8). Rats in group A were the control and were not exposed to any particular stress; Group B rats were made to swim in warm (34 ± 0.5 ^{o}C) water for 3 min/day; and Group C rats in warm (37 ± 0.5 ^{o}C) water for 6 min/day; while Group D rats were made to swim in warm (40 ± 0.5 ^{o}C) water for 12 min/day. These continued for three weeks, after which each group was divided into two Sub-Groups (n_{SG}=4). Rats in one subgroup from each of the four groups were sacrificed 24 hours after the last day of the 3 weeks of swim-stress, while the other sub-group from each of the four groups were left for 3 more weeks (to recover from the stress) before they were sacrificed.

Collection of Samples
Each rat was weighed before sacrificing by cervical dislocation, and blood samples were collected via cardiac puncture. Blood sample obtained from each rat was immediately transferred into EDTA bottle and mixed gently and thoroughly.

Collection of Data and Statistical Analysis
WBC, CD4 counts were determined using Improved Neubauer counting chamber and following the procedure documented by Chessbrough (1976). Field's stain A and Field's stain B were used for the Differential WBC.
The control and "Test groups" were compared using independent-samples t-test. The significant level was set to P value < 0.05.

RESULTS
The following results were obtained and are presented as mean ± SEM and level of significance is taken at "p value < 0.05" (*).

Weight Increase (g)
Comparing their final and initial weight showed that there was significant weight gain (P-value < 0.05) is in all the groups over the three or six weeks of the research. There was, however, no significant difference in weight gain of groups B C, and D_R compared to the control, while weight increase in D_{NR} had a significantly lower value when compared to the Control (Table 1) .

White Blood Cell Count (WBC), CD4, and Differential Count
The WBC was significantly reduced in both D_{NR} and D_R, while it was significantly higher in the B_{NR} and C_{NR} compared to the control. However, the WBC for B_R and C_R were not significantly different from that of the respective control (Table 2).

The CD4 count was significantly higher for B_{NR}, B_R, C_{NR} and C_R, while it was significantly lower in D_{NR} and D_R compared to the respective controls.

For the differential count, the percentages of WBC made up by neutrophils were significantly higher for D_{NR} and C_{NR}, but significantly lower for B_{NR}, while all the recovery groups showed no significant different in the percentages of neutrophils from those of the control (Table 2). An opposing trend was noted for the percentages of WBC made up by lymphocytes, such that significantly lower for D_{NR} and C_{NR}, but significantly higher for B_{NR}. The percentages for monocytes and basophils were, however, not significantly different throughout all the groups (Table 2).

The percentages of WBC made up by Eosinophils were found to be significantly (*P*-value < 0.01) lower in C_{NR}, but not significantly affected in other groups compared to the control (Table 2).

DISCUSSION
The significantly lower weight gain in D_{NR}, and the non-significant difference in the weight gain of all the subgroups of group B and C, and D_R (Table 1) is a telescope to the adverse effects of considerable amount of stress (i.e. sever and chronic stress) on living systems. Although, significant growth impairment cannot be used solely for a specific diagnosis, still it is a non-specific indicator of health problem, as it at least tells us that something is wrong somewhere in one of the body systems, perhaps in the immunological system as the case may be. Previous

publications of Wallace *et al*. (1995), Powera *et al*. (2000), Markowitz and Daum (2008), etc have earlier documented similar association between the different disease states and body weight loss/growth impairment.
The speculations from the gain in body weight/growth impairment (above) becomes more evident and better based by the significantly low WBC in both D_{NR} and D_R, the significantly high WBC in B_{NR} and C_{NR}; and the non-significant difference in the WBC of B_R and C_R compared to the control (Table 2). In other words, the significantly increased WBC in B_{NR} and C_{NR} is an indication that moderate stress could raise immune level, while the highly significantly lower WBC in D_{NR} indicates that severe stress has potential to adversely affect body immune level. The (considerably) significantly high CD4 count in B_{NR} and B_R, and the less significantly high CD4 count in C_{NR} and C_R (Table 2) can be linked to the activities of adrenergic stress hormones: cortisol, epinephrine, and norepinephrine. It is believed that when undergoing stress, the adrenal cortex releases the adrenergic stress hormones which increase the synthesis and release of cytokins, which consequently brings about the production of more white blood cells, more helper T-cells (CD4 cells), and thus boosts the immune system (Rassnick *et al*., 1994). This can explain the extremely high CD4 count in B_{NR} and B_R, since the adrenergic stress hormones (cortisol, epinephrine, and norepinephrine) released from the adrenal cortex (in response to the stress) would have augmented the production of white blood cells, more helper T-cells (CD4 cells), and thus boosts the immune system. One would normally expect even higher white blood cells and helper T-cells (CD4 cells) counts, and more boosts to the immune system in C_{NR} and D_{NR}, since more adrenergic stress hormones (cortisol, epinephrine, and norepinephrine) would be released from the adrenal cortex (in response to higher degree and much prolonged stress in groups C, and D). this was, however, not the case. The observed lower white blood cells and helper T-cells (CD4 cells) counts in C_{NR} and D_{NR} (Table 2) could be a surprise at first thought. But subsequent thoughts, and the fact that a high degree and prolonged stress leads to the build up of cortisol and other adrenergic stress hormones (not used up) in the body, would make this observation justifiable, since the build up of these unused adrenergic stress hormones is known to hinder the normal functioning of the immune system as well as reduced WBC and CD4 counts. In fact, previous works of Coren (2008) and Naliboff *et al*. (1991) among others had documented similar findings.

In a similar way, the withdrawal of the source of stress in the recovery groups caused the WBC and CD4 counts for each of the experimental (recovery) groups to return towards the values of the control groups (Table 2). This could be linked to the using up of the previously built up adrenergic hormones. It was however noted that these values (of WBC and CD4 counts in the recovery groups) could not totally return to the values of the control in the three weeks allowed for recovery. This observation raises an important speculation that the time it may require to completely recover (unaided) from an episode of stress upon stress withdrawal will most often be more than the actual duration of the stress, even though considerable recovery could still be accomplished within a recovery period that is of the same length as the duration of stress exposure. In other words, withdrawal of stress for three weeks might not just be enough to completely (and naturally) recover from a three-week episode of considerable stress, but could bring about the accomplishment of a considerable amount of recovery.

The significantly increased lymphocyte count in B_{NR}, and the significantly reduced lymphocyte count in C_{NR} and D_{NR} (Table 2) is parallel to other findings of this work and further establishes that moderate stress augments immunity while, severe stress brake it down, since the lymphocytes (and plasma cells) function mainly in connection with the immune system (Guyton and Hall, 2006). Dorian *et al*. (1982) and Kenji *et al*. (2000) have also documented similar trend for lymphocyte count with respect to exposure to stress. Dorian *et al*. (1982) found that there was stress-induced transiently elevated numbers of lymphocytes in eight psychiatry trainees taking their final oral fellowship examinations. For Kenji *et al*. (2000) surgical stress increases lymphocyte subsets and decreases the subsets that promote cellular immunity leading to cellular immunosuppression. This is parallel to many findings of this research.

However, neutrophils and lymphocytes were noticed to vary in opposite direction [Table 2], which is as well in support of the documentations of Sembulingam and Sembuligam (2006) as well as the findings of Kenji *et al*. (2000). In other words, neutrophils count was significantly reduced in B_{NR}, but significantly increased in C_{NR} and D_{NR}. This increase in neutrophils (a typical granulocytes) in the groups exposed to considerably severe stress presents another important (otherwise hidden) fact that even though considerably severe stress may significantly hinder normal immune functions, it, however, may not completely have adverse affects on all the body's defence lines/levels, since a typical granulocyte (neutrophils, in this case) which protects the body against invading

organisms mainly by ingesting them (that is, by phagocytosis), was significantly elevated in the rats exposed to considerably severe stress. However, this point would have being more solidified if other granulocytes (eosinophils, basophils) and monocytes in addition to neutrophils were as well significantly increased in C_{NR} and D_{NR} rather than just being comparable ($P > 0.05$) to those of the control. Perhaps a longer or more sever episode of stress would bring about such trend.

CONCLUSION

Stress, depending on the degree and duration, has both beneficial and suppressive effects on immunity; however, its either effects are often not permanent: they are reversible after its withdrawal. But the period it would take to completely and naturally recover (if at all possible) from the effects of an episode of stress is often more than the actual period of the stress. Even though, this research work establishes that stress could have either beneficial or detrimental effects (depending on the amount of the stress, and the duration of exposure to the stress), and that either of the effects is considerably reversible, we are at present not able to determine the specific amount of stress that is beneficial, and that which is detrimental. A better understanding of the amount of stress that is beneficial, and that which is detrimental could be determined if successive research could focus on estimation of stress and the derivation of a standard unit that could be used universally for the amount of stress in both humans and experimental animals.

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Table 1: Weight Increase (g) after the 10 Weeks of Research

	Control		Group B		Group C		Group D	
	NR	R	B_{NR}	B_R	C_{NR}	C_R	D_{NR}	D_R
Weight before sacrifice (g)	214.00 ± 3.24	217.50 ± 2.33	212.50 ± 5.25	213.00 ± 3.56	210.50 ± 4.33	213.75 ± 2.50	202.75 ± 0.85**	212.75 ± 2.95
Initial weight (g)	191.25 ± 2.29	192.00 ± 3.11	191.75 ± 6.09	191.50 ± 7.79	192.75 ± 6.86	192.50 ± 6.33	191.25 ± 1.44	191.75 ± 3.40
Weight increase (g)	22.75 ± 1.93	25.50 ± 2.96	20.75 ± 2.36	21.50 ± 6.17	17.75 ± 4.61	21.25 ± 6.85	11.50 ± 2.18 **	21.00 ± 1.08

Data are expressed in mean ± SEM.
** = "*P*-value < 0.01" indicated significant difference when compared with the control group by independent samples t test

Table 2: Comparison of White Blood Cell Count (WBC), CD4, Percentage of White Blood Cells that is Neutrophils (N), Lymphocytes (L), Monocyte (M), Eosinophil (E) and Basophil (B)

	Control		Group B		Group C		Group D	
	NR	R	B_{NR}	B_R	C_{NR}	C_R	D_{NR}	D_R
WBC (cells/uL)	2270 ± 105.4	2195 ± 102.1	4325 ± 197.7***	2325 ± 51.88	2790 ± 78.52**	2260 ± 72.57	1475 ± 26.30***	1880 ± 68.00*
CD4 (cells/uL)	655.0 ± 17.08	645.0 ± 12.58	890.0 ± 50.66**	715.0 ± 9.57**	715.0 ± 9.57*	690.0 ± 12.91*	500.0 ± 8.17***	565.0 ± 18.93**
N (%)	58.50 ± 0.65	59.25 ± 1.31	55.5 ± 0.87*	58.25 ± 1.49	61.75 ± 1.25*	58.5 ± 0.65	64.75 ± 1.44**	60.5 ± 1.04
L (%)	30.25 ± 0.48	29.75 ± 0.75	32.00 ± 0.58*	30.25 ± 0.48	27.75 ± 0.48**	28.75 ± 1.03	26.25 ± 0.85**	28.25 ± 1.25
M (%)	7.00 ± 0.41	6.75 ± 0.63	7.50 ± 1.19	7.00 ± 1.08	7.25 ± 0.75	7.50 ± 0.50	6.25 ± 0.25	6.75 ± 0.63
E (%)	3.50 ± 0.29	3.25 ± 0.48	2.75 ± 0.75	2.25 ± 0.25	2.00 ± 0.57*	3.00 ± 0.71	2.00 ± 0.71	2.75 ± 0.25
B (%)	0.75 ± 0.25	1.00 ± 0.41	2.75 ± 0.85	2.25 ± 0.75	1.25 ± 0.25	2.25 ± 0.63	0.75 ± 0.75	1.75 ± 0.63

Data are expressed in mean ± SEM.
* = "*P*-value < 0.05", ** = "*P*-value < 0.01", *** = "*P*-value < 0.001" indicated significant difference when compared with the control group by independent samples t test.

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